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AETIOLOGY OF FUNGAL PATHOGENS OF GARDEN EGG (*SOLANUM MELONGENA* L. JUSS) IN NSUKKA AREA

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ABSTRACT

Post-harvest diseases of garden egg (*Solanum melongena* L.) were investigated in Nsukka, Enugu State, Nigeria. Fruits of a common local variety: *Solanum melongena var. Esculentum* showing symptoms of rots were collected from three local markets within Nsukka. Isolations made from diseased tissues were inoculated into potato Dextrose Agar (PDA) plates. Pure cultures were obtained by several aseptic transfers of colony growth from PDA plates to clean PDA plates. Identifications of isolated fungal pathogens were made based on cultural characteristics and microscopic examinations of the cultures. The results implicated three fungal species: *Mucor sp., Rhizoctonia solani* and *Aspergillus niger*. The pathogenicity of the isolated fungi was confirmed. The antifungal effect of extracts of Medicinal plants on these pathogens is still in progress.

KEYWORDS: Aetiology, Pathogens, Post-Harvest, *Solanum melongena*, Extract

INTRODUCTION

Garden egg (*Solanium melongena* L.) family *solanaceae* is a curious kind of fruit used for many purposes among which are to achieve weight loss within a short period, to eliminate unnecessary salt in maintaining proper functioning of the heart, to reduce the sugar content level in diabetics because of its low calorie content and to reduce blood cholesterol. *Solanum melongena* fruit is also an important nutritive plant but a very perishable vegetable with a short shelf-life. It is susceptible to fungal diseases caused by *Phytophthora nicotianea,* var. parasitica and if the fruit touches the ground, *Corticium rolfsir* will cause an infection.

Many fruits and vegetables are perishable especially in tropical and subtropical regions without adequate refrigeration (Coursey, 1983). The magnitude of post-harvest losses in fresh fruits and vegetables is estimated to be 25.80% (Thrupathi *et al*., 2006). Most of the product is lost after the harvest because of inadequate handling and preservation methods (Wills *et al.*, 1981; Liu and Ma, 1983). People in developing counties often cannot afford the use of cold storage facilities (Liu and Ma, 1983), which may be because of lack of capital or lack of technical knowledge of small scale growers and retailers in these areas (Pantastico and Baustista, 1976).

Fungi are the most important and prevalent pathogens infecting a wide range of host plants and causing destructive and economically important losses of most fresh fruits and vegetables during storage and transportation (Sommer, 1985). Various strategies that have been used to control the pathogens include the use of chemical and biological agents (Neergaard, 1977). Some chemicals are generally considered toxic to mammals including man. These problems associated with the use of synthetic chemicals necessitate the use of anti-microbial agents which are cheap, easier to prepare and that would be available for the control of post-harvest fungal diseases of plant. Some plant extracts are specific, they are biodegradable, cheap, readily available and environmentally safer than synthetic chemicals. Extracts of plants such as *Azadirachta indica* are believed to be efficacious in the control of plant fungal diseases.

Fruits vary in their innate resistance to decay and are most resistant when relatively dry and firm. When fruits are harvested they have limited post-harvest life. They no longer receive water or nutrition from the plant. Naturally occurring senescence in produce leads to softening of the tissues and often loss of preformed antimicrobial substances. These changes in the fruits make them less desirable to consumers (Bartz *et al.,* 2009). The improper handling, packaging, storage and transportation may result because of the changing physiological state of the fruits and vegetables (Wilson *et al.,* 1991). Fruits due to their low pH, higher moisture content and nutrient composition are very susceptible to attack by pathogenic microorganisms, which cause rots and produce mycotoxins (Moss, 2002).

Schwartz and Gent (2007) reported that several pathogens are responsible for post-harvest rot of fruits and vegetables; which include pathogenic fungi such as *Sclerotinia sclerotiorum, Penicilium* spp., *Rhizoctonia solani*, *Phytophthora* spp., *Pythium* spp., *Rhizopus* spp. and several bacteria such as *Erwinia carotovora* and *E. chrysanthemi.* Naureen *et al.* (2009) also listed a number of fungi such as *Alternaria alternate, Aspergillus* spp., *Fusarium* spp., *Phytophthora capsici* and *Rhizopus stolonifer* as causative agents of post-harvest diseases of fresh fruits and vegetable.

Whitaker (1990) reported that plant pathogenicity and spoilage of fruits and vegetables by rotting are manifestations of pectinolytic enzymes activity. These pectinases are commercially sourced from fungi (Singh *et al*., 1999). The enzymes act on pectic substances which are high in molecular weight, negatively charged, acidic complex glycosidic polysaccharides that are present as the calcium and magnesium pectates (Rastogi, 1998).

Sakai *et al.,* (1993) reported that the pectic substances account for 0.5 – 4.0% of the fresh weights of plant material, including fruits. These pectinolytic enzymes are widely distributed among fungi, bacteria and many types of yeast; specifically *Aureobasidium pullulans* and *Rhizoctonia solani* Kuhn (Marcus *et al., 1986)* have been named among others.

The quality of garden egg is affected by post-harvest handling, packaging, transportation and storage which may result in decay and production of micro-organisms which become activated because of the changing physiological state of the fruit (Wilson et al., 1991). This work is being undertaken specifically to isolate and identify post-harvest fungal, pathogens of *solanum melongena* L. Juss with a view of determining the effect of crude leaf extract of *A. indica* in the control of the pathogens.

MATERIALS AND METHODS

Materials

The garden egg varieties (green and white) showing symptoms of rots were collected from three local markets within Nsukka, Enugu State, Nigeria.

METHODS

Sterilization

Materials such as agar were aseptically sterilized in an autoclave at 103 KN M^{-2} and 121^0C for 15 minutes; Petri dishes, beakers, test tubes, filter papers and metallic materials such as spatula and forceps were sterilized using hot air oven at a temperature of 160^oC for 1 hour. The wire loops were also sterilized until red hot and allowed to cool before using 70% alcohol to wipe the work tops to prevent contamination.

Preparation of Culture Media

All culture media were prepared according to instructions by manufacturers and autoclaved at 121^oC for 15 minutes.

Isolation of Fungal Isolates

The isolation technique used was the same as that used by Chiejina (2008). Thin sections (2mm diameter) were cut from the periphery of diseased garden egg fruits and sterilized in 0.1% mercuric chloride for 2 minutes. These sections were rinsed in 3 changes of sterile distilled water and plated in Potato Dextrose Agar (PDA) plates. The plates were incubated at room temperature ($27^oC + 2^oC$) for 7 days. Pure cultures were obtained by several transfers of the colony growth from PDA plates to clean PDA plates aseptically.

Pathogenicity Tests

A pathogenicity tests were carryout using techniques of Okigbo *et al*. (2009). Healthy fruits of garden egg were washed in sterile distilled water and surface sterilized with 0.1% mercury chloride solution. With the aid of a sterile cork borer, a cylindrical core was removed from the garden egg fruits. A pure culture of the isolate was introduced into the open core and the core was replaced and sealed with sterile petroleum jelly. The fruits were kept at room temperature for 7-10 days. On establishment of disease symptoms, inoculums from the infected fruits were taken and cultured. Pure cultures were identified according to (Barnett and Hunter, 1999; Alexopoulos *et al.*, 2002). The symptoms were identical to those of naturally infected garden egg. Morphological characteristics of conidia and mycelia of the fungi that were re isolated from inoculated fruits confirmed Koch's postulates.

Identification

Identification of the fungi was based on the growth patterns, colour of mycelia and microscopic examinations of vegetative and reproductive structures according to (Barnett and Hunter, 1999; Alexopoulos *et al*., 2002).

RESULTS

Fungi isolated were identified as *Mucor* sp., *Rhizoctonia solani* and *Aspertillus niger*. Pathogenicity tests confirmed that, the fungi isolated were the causal agents of the rot. The morphological features and vegetative structures of all the isolates are shown in plates 1, 2, and 3. In this study, *Mucor* ramossisimus., *Rhizoctoma solani* and *Aspergillus niger* have been implicated in post-ahrvest fungi diseases affecting garden egg fruits. *Aspergillus* spp belong to the class Deutoromycetes. Spore sizes is (about 2-5cm). Aspergillus spp appear black on PDA. *Rhizoctonia solani* belongs to the class *Agaricormycetes* in the family *corpicsacease.* The spores of the *Rhizoctonia solani* on PDA was dark brownish with white patches at the head. The grown mucor spp colonies on PDA were white at first turning brownish to grey with age, and become heavily specked with the appearance of sporangia.

PERCENTAGE FREQUENCY OF OCCURRENCE OF FUNGI (PATHOGENS) ON THE DISEASED GARDEN EGG FRUITS

The percentage of fungal occurrence was done to determine the frequency of occurrence of the different fungal isolates. Isolated pathogens from different garden egg fruits that were found diseased were cultured differently. The number for each of the isolates in the eight different fruits were recorded, calculated and expressed as percentage.

$$\text{Percentage of occurrence} = \frac{X \times 100}{1}$$

Where X = Total number of each organism in all the fruits.

N = Total number of the entire organisms in all the fruits screened

Table 1: Percentage Frequency of Occurrence of Fungi on Diseased Garden Egg Fruits

Isolates	No of Isolation	Frequency of Occurrence (%)
Mucor	11	26.82
Aspergillus niger	14	34.14
Rhizoctonia solani	16	39.02

Diseased Severity Rating: The Degree of Decay of the Fruits by the Pathogens was Assessed Using a Diseased Rating of 1 to 5 (Eze and Maduewesi, 1990).

Where: 1 =No decay, 2 = slight decay (10-30%), 3 = moderate decay, (31-60%), 4 = severe decay (61-90%) and 5 = complete decay (91-100%)

RESULTS

Table 2

Days	1	2	3	4	5	6	7	8	9	10
Fungal Isolates	**Severity Ratings**									
Mucor ramossisimus	1	2	2	2	2	2	3	3	4	4
Aspergillus niger	1	2	2	3	3	3	3	4	4	5
Rhizoctonia solani	1	2	2	3	3	3	4	4	5	5
Control	1	1	1	1	1	1	1	1	1	1

Disease Severity Rating: Table 2, shows the disease severity ratings on the garden egg fruits. It was found that *Rhizoctonia solani* was the most severe pathogen on the fruits as severe decay occurred on 4th day of incubation and *Mucor* ramossisimus Was the least severe.

DISCUSSIONS

Three fungal pathogens were isolated from the garden egg fruits indicating that these pathogens could grow and survive in garden egg fruits. The results of this investigation shows that Garden egg fruits are prone to infections by a variety of fungal pathogens. These pathogens; *Aspergillus niger*, *Rhizoctonia solani* and *Mucor* ramossisimus produce numerous air-borne sessile spores that can easily land on the fruits while on display in the markets for sale. These organisms might have gained entry through stomatal openings, growth cracks or surface injuries (Wills et al., 1981). There it is possible that possession of nutrients suitable for the growth of pathogen by garden egg facilitated the infections by these organisms.

They can grow and survive on garden egg and were the causal agents of post-harvest in Nsukka, Enugu State of Nigeria. The high frequency of occurrence of the fungi on the fruits investigated confirmed their virulence on the fruits. The pathogens could have gained entry through wounds or bruises created during harvesting and packaging of the fruits for delivery to the markets. Their growth is probably due to the fact that garden egg fruits contain some nutrients suitable for the pathogen to grow. The spoilage of the fruits during post-harvest storage is due to infection by these micro-organisms which may be as a result of rough handling, poor road and storage facilities (Liu and Ma,1967).

Losses caused by post-harvest diseases have increased recently than generally realized because the value of fruit and vegetables has increased (Eckert and Sommer, 1967). Splittoesser, (1987) reported that species of *Penicillium*, *Aspergillus* and *Mucor* spp are common post-harvest fungi. Also, *Aspergillus niger, A. tamarii*, *Mucor* spp, *penicillium oxalicum*, and *P. digitatum* were found to cause rot of cassava (Okigbo *et al*., 2009). *Mucor* ramossisimus, *Rhizoctonia solani* and *Aspergillus niger* were implicated as pathogens when tested on healthy products. *Mucor* species were of relatively minor impotance as post-harvest In the last two decades, however, they have caused serious decay of Strawberries,Pears,Apples, Guavas,Tomato and Sweet potato (Michailides and Spotts,1990).

Certain environmental factors favour the growth of these pathogens on the garden egg fruits. They include temperature, high humidity especially when fruits are wrongly packed and wind that may have carried the light spores of *Mucor* spp. and *Rhizoctonia solani* from any diseased vegetables and fruits.(Kunimoto *et al.*,(1977), since they are sold in close proximity with other vegetables and fruits in the markets.

In spite of the heavy crop loss due to rot caused by these pathogens, they also cause serious health problems to man. Some of the moulds from these pathogens could produce mycotoxins on fruits (Stinson *et al*., 19). Kurup (2003) stated that these pathogenic fungi on the other hand could cause infections or allergies in susceptible individuals. Rhizopus spp. and Mucor spp. have been shown to cause *Mucorosis* in the immune system of man (Alverez and Nishijima, 1987). The toxins from these fungi cause respiratory and ulceration diseases in human beings.Some *Alternaria* and *Mucor* spp. in dust are known to cause irritation to asthma and allergy sufferers.

The most serious post-harvest diseases are those that cause rapid and extensive breakdown of crops that are high in moisture content and nutrients. It has been estimated by Liu and Ma (1983) that, 30% of all fruit rots are caused by a species of penicillium. In this work, *Aspergillus* spp and *Rhizoctonia solani* are the most virulent pathogens.

Mucor : Fluffy

MUCOR* RAMOSSISIMUS:* Fluffy dirty white mycelia seen on both infected garden egg fruits and in PDA Plates. Microscopic examinations of the ccultures showed sporangiophores with globose sporangia at their tips but with no rhizoids at their bases .

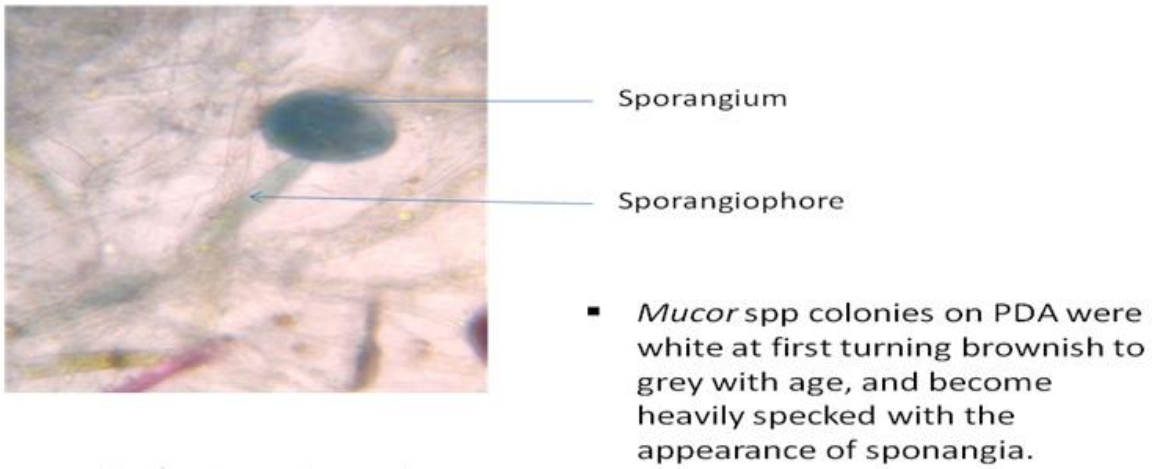

Figure 1: Photomicrograph of *Mucor* Spp (mg x400)

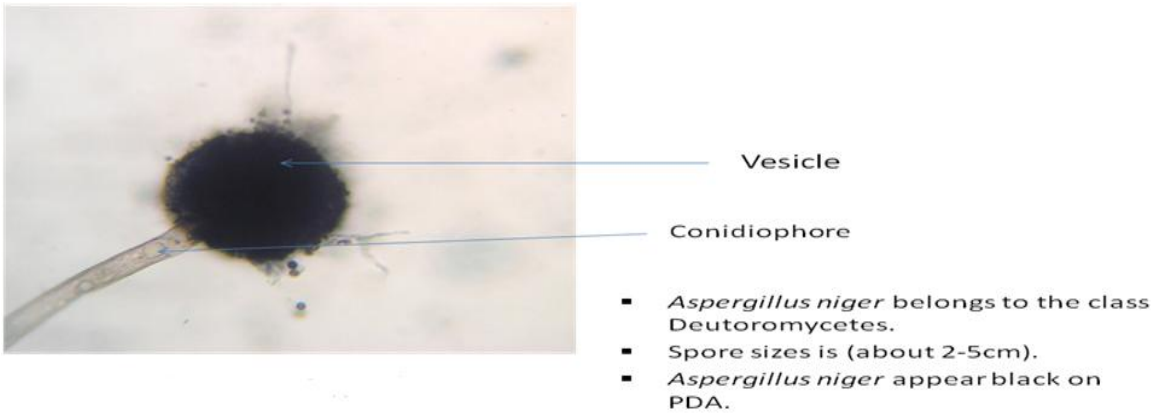

Figure 2: Photomicrograph of *Aspergillus niger* (mg x400)

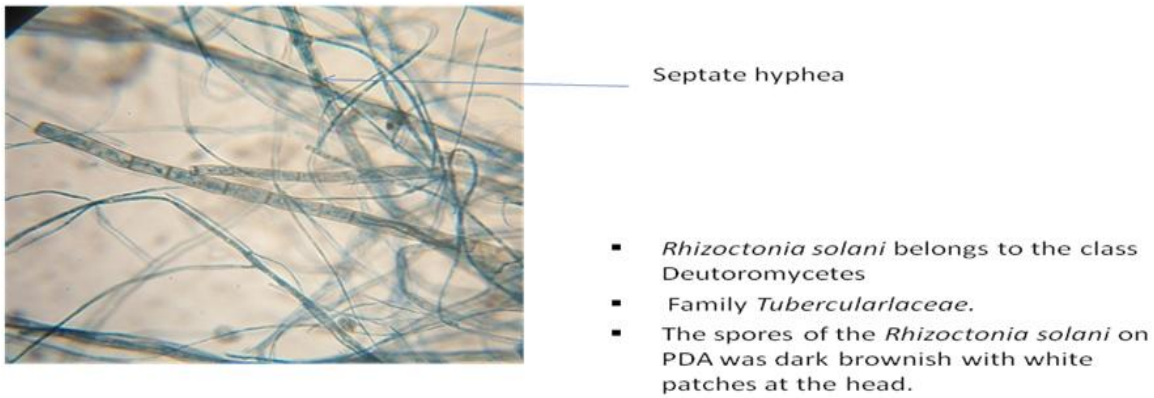

Figure 3: Photomicrograph of *Rhizoctonia Solani* (mg x400)

Fruit rot can occur from the fruit-set to harvest period. It is impossible to completely eliminate post-harvest losses, but it is possible and better to reduce them by 50% to 60% (Kader, 2002). Reducing post-harvest loss of fruits that have already been produced is more sustainable and environmentally sound than increasing production areas to compensate for these losses (Kader, 2002).

CONCLUSIONS

From this study, it can be safely adduced that *Aspergillus niger*, *Rhizoctonia solani*, and *Mucor ramossisimus* are the causal organisms responsible for the post –harvest rot of *Solanum melongena (Garden egg).*Work is therefore, in progress for the use of extracts from medicinal plants as antifungal agents against the isolated and identified pathogens.

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